

METABOLISM OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP) IN JUVENILE AND FETAL MARMOSET AND RAT.

Y. Kurata¹, F. Makinodan¹, N. Shimamura¹, M. Okada¹, M. Katoh¹.

¹Mitsubishi Chemical Safety Institute Ltd., Ibaraki, Japan

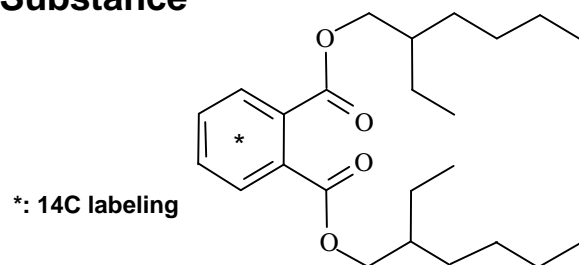
DEHP is most widely used as a plasticizer of polyvinyl chloride in the manufacture of a wide variety of consumer products. The general population is exposed to DEHP in food, water, and air, and the largest source of exposure is dietary (Kavlock et al., 2002). DEHP induces testicular lesion in juvenile rats, however, clear species difference was seen in that effect. In the long-term and high-dose toxicity study (15-month treatment, at 100 to 2500 mg/kg/day) in juvenile marmoset, no obvious testicular toxicity was observed (Kurata et al., SOT 2003). Clear species difference is also seen in its metabolism, and the metabolites such as mono-ester (MEHP) and oxidized MEHP were suspected to be a cause of testicular lesions. Then, We compared the metabolic profiles of DEHP in marmoset with that in rats under the same condition.

STUDY-I: Radioactivity in the plasma, urine, feces, and tissues were compared together with metabolite contents when ¹⁴C-DEHP was singly dosed to juveniles by gavage.

STUDY-II: Tissue distribution in fetuses was compared when ¹⁴C-DEHP was singly dosed to dams on gestation day of 130 for marmosets and day of 20 for rats.

MATERIALS & METHODS

Test Substance



Study –I (study in juvenile animals)

Test animals

- | | |
|-------------|--------------------------------------|
| - Marmoset: | Common marmoset
(3 months of age) |
| - Rat: | Crj:CD(SD)IGS
(4 weeks of age) |

Dose level (single dose by oral gavage)

100 mg/kg (10 MBq/kg)

Examinations

- | | |
|--------------------------------|--------------------------------|
| - Plasma radioactivity: | 2, 4, and 8 hours after dosing |
| - Urine & feces radioactivity: | 0 to 24 hours after dosing |
| - Tissue radioactivity: | 8 and 24 hours after dosing |
| - ARG: | 24 hours after dosing |
| - Profile of metabolites: | Plasma, urine, and feces |

Study –II (study in pregnant animals)

Test animals

- | | |
|-------------------------|--|
| - Marmoset dam & fetus: | Common marmoset
(130 days of gestation) |
| - Rat dam & fetus: | Crj:CD(SD)IGS
(20 days of gestation) |

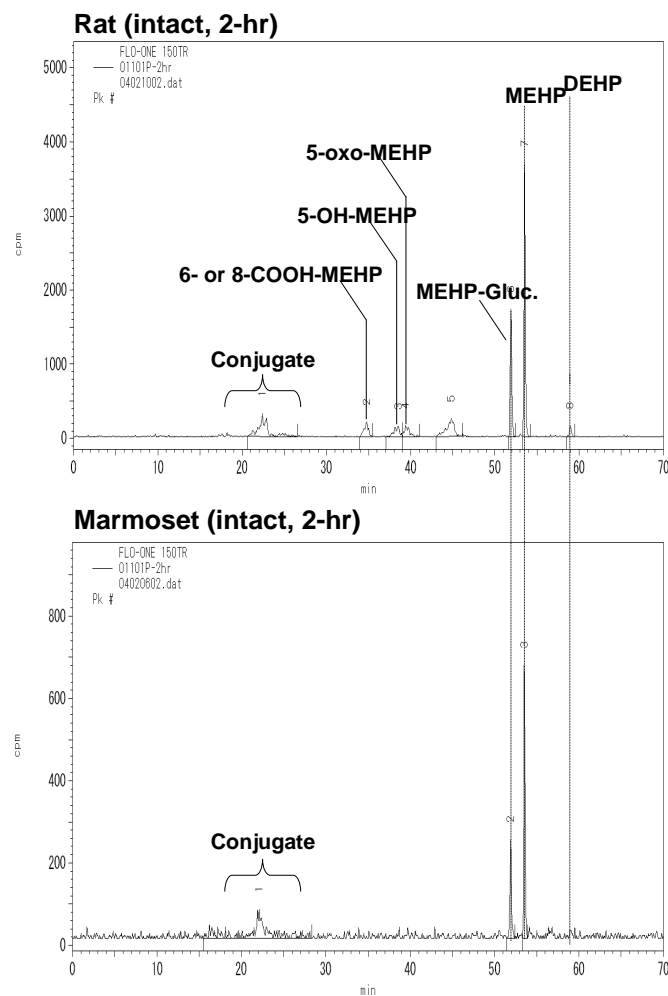
Dose level (single dose by oral gavage to dams)

100 mg/kg (50 MBq/kg)

Examinations

- | | |
|----------------------------------|-----------------------|
| - Tissue radioactivity in fetus: | 24 hours after dosing |
| - ARG of fetus: | 24 hours after dosing |

P-1. Plasma Radioactivity and Contents of DEHP Metabolites (Study-I in Juvenile Animals)



Rat		(Mean \pm S.D., n=3)		
Metabolite		μg equivalent of DEHP/mL		
		2-hr	4-hr	8-hr
Radioactivity		52.51 \pm 6.62	34.85 \pm 6.54	16.97 \pm 3.76
Unknown PA		9.44 \pm 2.48	8.24 \pm 1.57	3.90 \pm 0.60
COOH-MEHP		3.73 \pm 0.49	2.58 \pm 0.55	1.03 \pm 0.16
OH-MEHP		2.98 \pm 0.43	2.52 \pm 0.57	1.02 \pm 0.32
OXO-MEHP		2.91 \pm 0.49	2.50 \pm 0.39	1.90 \pm 0.68
OH-MEHP		6.72 \pm 0.43	6.63 \pm 0.68	5.67 \pm 0.68
MEHP-Gluc.		8.50 \pm 2.28	3.59 \pm 1.45	0.84 \pm 0.39
MEHP		17.49 \pm 2.81	8.79 \pm 2.43	2.61 \pm 1.35
DEHP		0.74 \pm 0.57	N.D.	N.D.

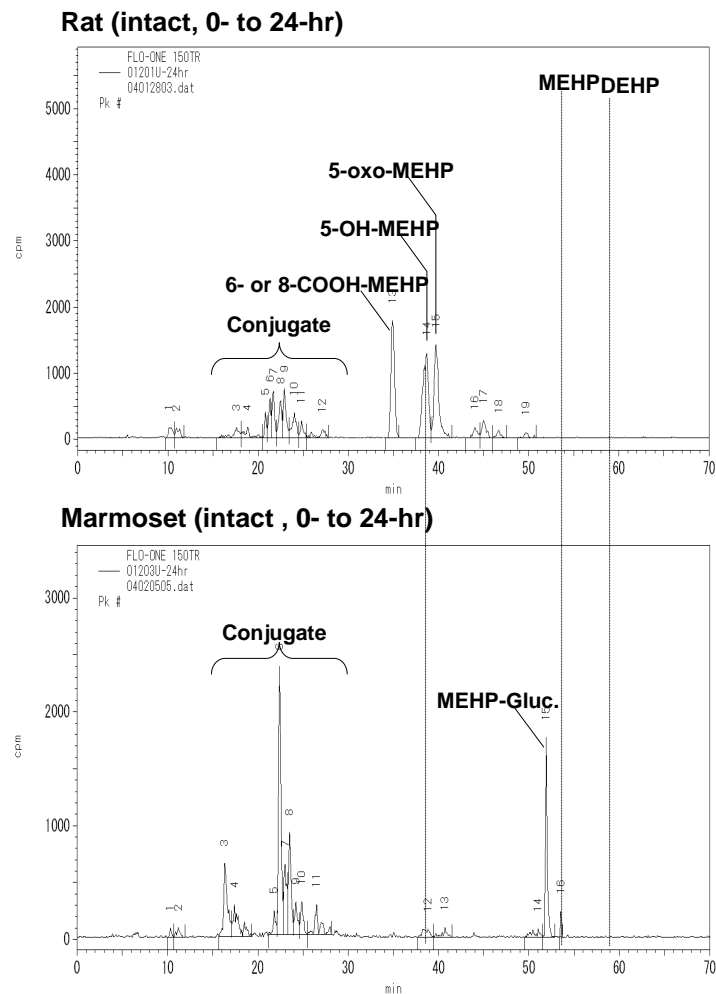
N.D.: not detected

Marmoset		(Mean \pm S.D., n=3)		
Metabolite		μg equivalent of DEHP/mL		
		2-hr	4-hr	8-hr
Radioactivity		3.32 \pm 5.37	0.87 \pm 1.26	0.16 \pm 0.19
Unknown PA		4.34*	N.A.	N.A.
MEHP-Gluc.		1.47*	N.A.	N.A.
MEHP		3.71*	N.A.	N.A.
DEHP		N.D.	N.A.	N.A.

*: n=1, N.D.: not detected, N.A.: not analyzed

by Radio HPLC & LC/MS/MS analysis

P-2 . Urinary Radioactivity and Contents of DEHP Metabolites (Study-I in Juvenile Animals)



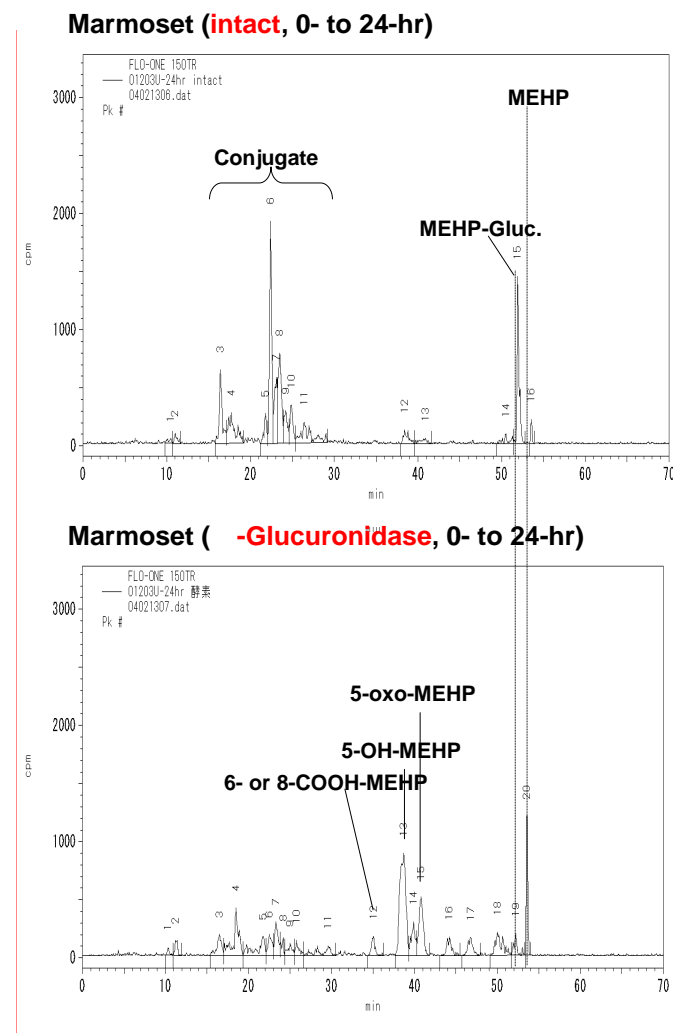
Time (hr)	Marmoset ^{*1}		Rat ^{*1}	
	[8.18% of dose in urine]		[58.04% of dose in urine]	
	Metabolite	% of dose	Metabolite	% of dose
0 - 24	(M-UM-01)	N.D.	(R-UM-01)	1.04
	(M-UM-02)	N.D.	(R-UM-02)	1.03
	(M-UM-03)	N.D.	(R-UM-03)	1.48
	(M-UM-04)	N.D.	(R-UM-04)	1.61
	(M-UM-05)	N.D.	(R-UM-05)	1.18
	(M-UM-06)	0.25	(R-UM-06)	2.03
	(M-UM-07)	2.04	(R-UM-07)	3.51
	(M-UM-08)	N.D.	(R-UM-08)	2.86
	(M-UM-09)	1.01	(R-UM-09)	3.35
	(M-UM-10)	0.49	(R-UM-10)	1.52
	(M-UM-11)	N.D.	(R-UM-11)	1.17
	(M-UM-12)	N.D.	(R-UM-12)	1.24
	(M-UM-13)	N.D.		
	COOH-MEHP (M-UM-14)	N.D.	COOH-MEHP (R-UM-13)	10.66
	OH-MEHP (M-UM-15)	0.31	OH-MEHP (R-UM-14)	10.66
	OXO-MEHP (M-UM-16)	N.D.	OXO-MEHP (R-UM-15)	10.30
	OH-MEHP (M-UM-17)	N.D.	OH-MEHP (R-UM-16)	0.90
	OH-MEHP (M-UM-18)	N.D.	OH-MEHP (R-UM-17)	2.14
	OXO-MEHP (M-UM-19)	N.D.	OXO-MEHP (R-UM-18)	0.73
	(M-UM-20)	N.D.	(R-UM-19)	0.61
	MEHP-Gluc. (M-UM-21)	1.16		
	MEHP (M-UM-22)	0.26		

*1: n=3

N.D.: not detected

by Radio HPLC & LC/MS/MS analysis

P-3. -Glucuronidase Treatment of Marmoset Urine (Study-I in Juvenile Animals)



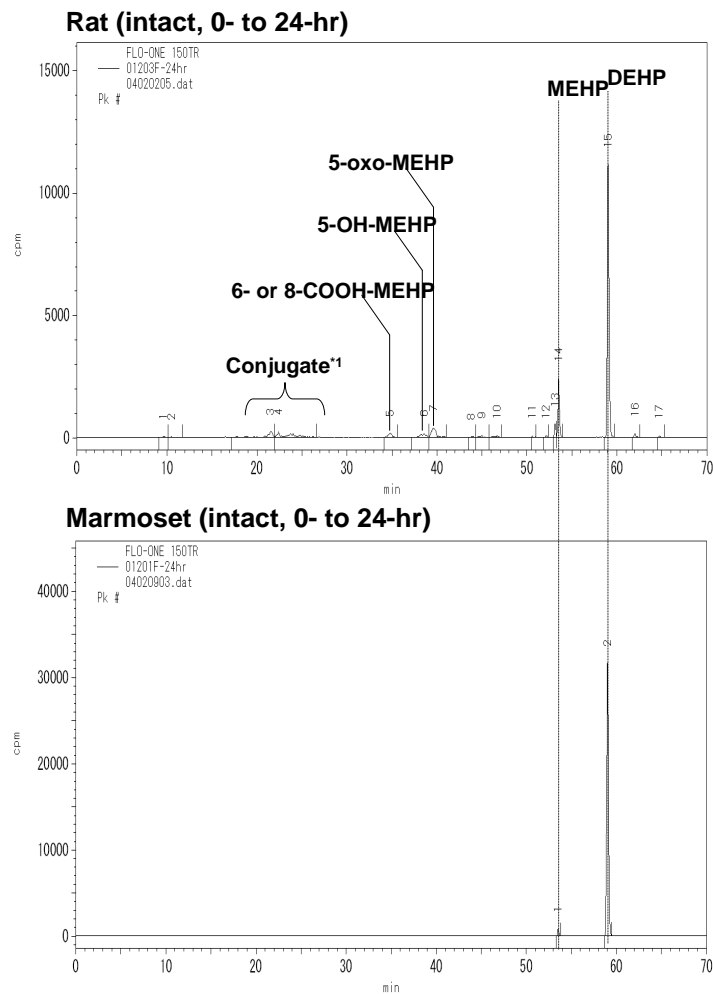
Time (hr)	Metabolite	[19.45% of dose in urine]*	
		% of dose	
		Intact	Enzymatic treatment
	(M-UM-01)	0.14	0.13
	(M-UM-02)	0.25	0.34
	(M-UM-03)	1.78	0.75
	(M-UM-04)	1.60	1.75
	(M-UM-05)	N.D.	N.D.
	(M-UM-06)	0.64	0.47
	(M-UM-07)	4.03	0.57
	(M-UM-08)	1.50	0.77
	(M-UM-09)	2.54	0.13
	(M-UM-10)	0.88	0.20
	(M-UM-11)	0.89	0.34
	(M-UM-12)	0.98	0.43
	(M-UM-13)	N.D.	N.D.
0 - 24	COOH-MEHP (M-UM-14)	N.D.	0.57
	OH-MEHP (M-UM-15)	0.54	4.94
	OXO-MEHP (M-UM-16)	N.D.	1.04
	OH-MEHP (M-UM-17)	0.32	2.01
	OH-MEHP (M-UM-18)	N.D.	0.72
	OXO-MEHP (M-UM-19)	N.D.	0.74
	(M-UM-20)	0.41	1.24
	MEHP-Gluc. (M-UM-21)	2.67	0.54
	MEHP (M-UM-22)	0.30	1.78

*: n=1

N.D.: not detected

by Radio HPLC & LC/MS/MS analysis

P-4. Fecal Radioactivity and Contents of DEHP Metabolites (Study-I in Juvenile Animals)



Time (hr)	Marmoset [*]		Rat [*]	
	[49.67% of dose in feces]		[28.54% of dose in feces]	
	Metabolite	% of dose	Metabolite	% of dose
0 - 24	(M-FM-01)	N.D.	(R-FM-01)	0.28
			(R-FM-02)	0.22
			(R-FM-03)	2.42
			(R-FM-04)	3.23
			COOH-MEHP (R-FM-05)	1.23
			OH-MEHP (R-FM-06)	1.26
			OXO-MEHP (R-FM-07)	2.06
			OH-MEHP (R-FM-08)	0.21
			OH-MEHP (R-FM-09)	0.51
			OXO-MEHP (R-FM-10)	0.37
			(R-FM-11)	0.11
			MEHP-Gluc. (R-FM-12)	0.32
			(R-FM-13)	0.47
	MEHP (M-FM-02)	2.78	MEHP (R-FM-14)	2.58
	DEHP (M-FM-03)	46.89	DEHP (R-FM-15)	12.96
			(R-FM-16)	0.23
			(R-FM-17)	0.09

^{*}: n=3
N.D.: not detected

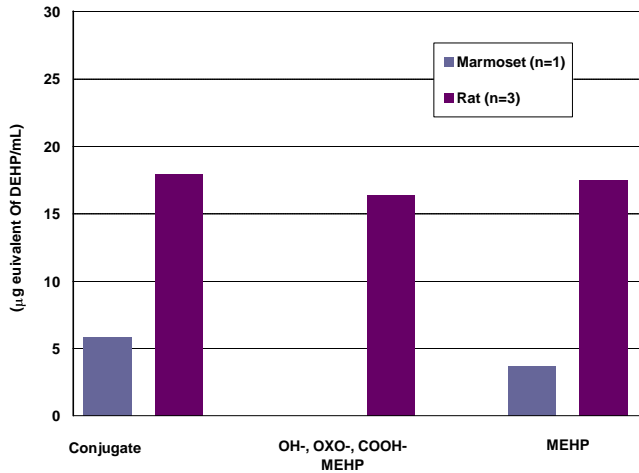
by Radio HPLC & LC/MS/MS analysis

P-5. Summary of DEHP Metabolism (Study-I in Juvenile Animals)

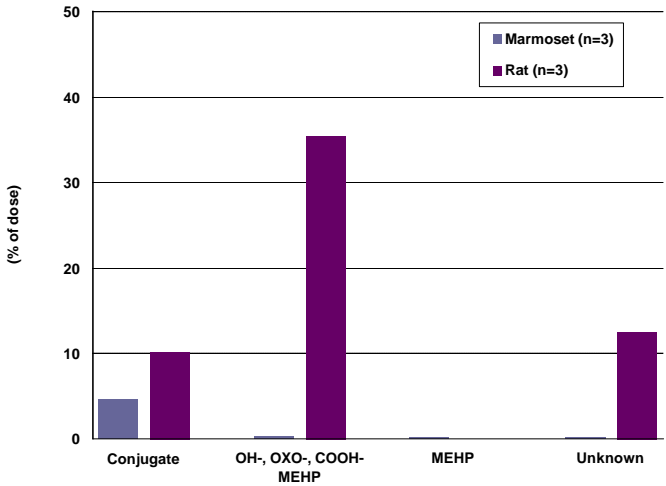
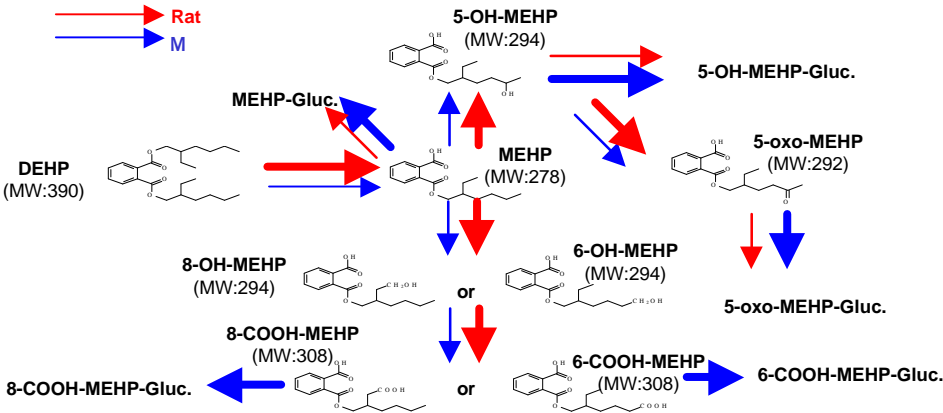
Urine & Feces Excretion of Radioactivity in Juvenile Animals

(Mean ± S.D.)					
	Route	Time	% of dose		
			Urine (+ Cage washing)	Feces	Total
Marmoset	P.O.*1 100 mg/kg	0-24hr	12.83 ± 15.05	49.67 ± 29.12	62.50 ± 40.07
	I.V.*2 10 mg/kg	0-24hr	44.79	15.23	74.84
F.=12.83/44.79*100=28.6%					
(Mean ± S.D.)					
	Route	Time	Cumulative excretion of radioactivity (% of dose)		
			Urine (+ Cage washing)	Feces	Total
Rat	P.O.*1 100 mg/kg	0-24hr	61.59 ± 9.27	28.54 ± 17.05	90.13 ± 8.28

*1: n=3, *2: n=1

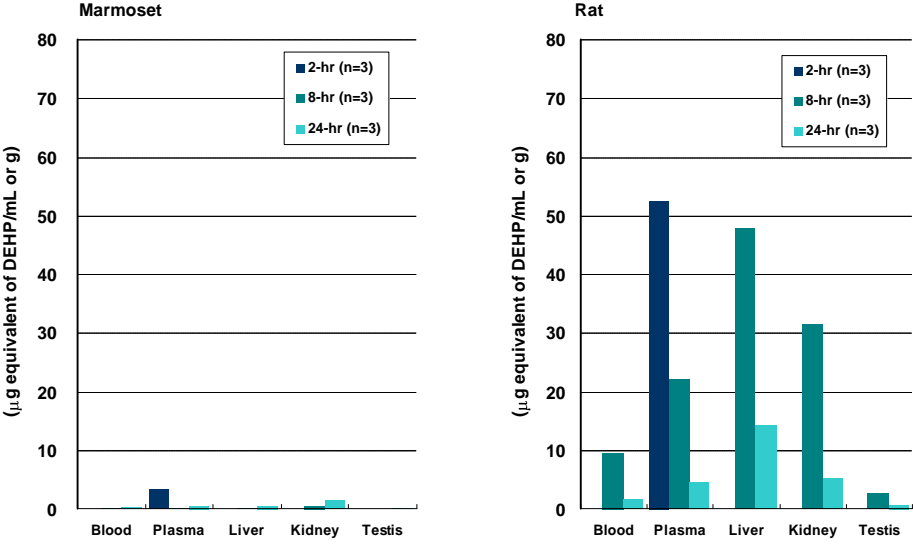


Metabolites in Plasma



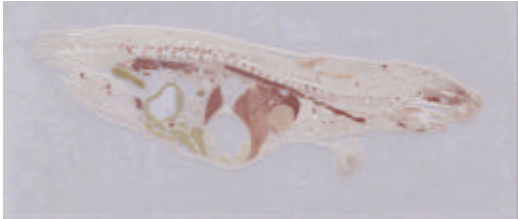
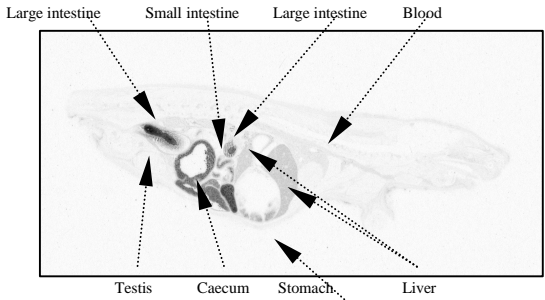
Metabolites in Urine

P-6. ARG and Tissue Distribution of Radioactivity (Study-I in Juvenile Animals)

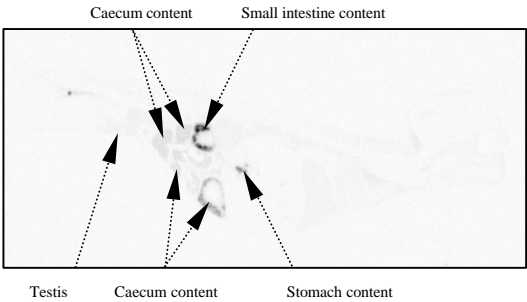


Concentration of Radioactivity in Tissues of Juvenile Animals

Rat (24-hr)



Marmoset (24-hr)



Testis

RESULTS & CONCLUSION

Study –I (in Juvenile Animals)

- Three types of metabolites such as (1) MEHP, (2) conjugates of MEHP and MEHP-metabolites, or (3) unconjugated MEHP-metabolites such as 5-OXO, 5-OH, and 6 or 8-COOH MEHP were detected in rat plasma. However, unconjugated MEHP-metabolites were not detected in marmosets. (P-1, P-5)
- As for rats, large amount of unconjugated MEHP-metabolites (about 35% of dose), which were the same kinds of plasma, were detected in the urine. However, as for marmosets, the majority was conjugated MEHP and its metabolites. (P-2, P-5)
- About 60 % of the dose was excreted into the urine in rats. As for marmoset, although the bioavailability was presumed to be about 20 to 30%, the majority of the dose was excreted into the feces. (P-3, P-4, P-5)
- Specific accumulation in the testes was noted neither in rats nor in marmosets. However, the radioactivity in marmoset liver, kidney, or testes was much lower than those of rats. (P-6)

Study –II (Study-II in Pregnant animals)

- Specific accumulation in the testis was noted in neither rats nor in marmosets, although high radioactivity was observed in the digestive tract, liver, and kidney. The radioactivity in rat testis was about 20-times higher than that in marmosets. (P-7)

Clear species differences in plasma and tissue radioactivity concentrations, and in the content of metabolites were demonstrated, and that might be the causes of species difference in testicular lesions.